SOPHION BIOSCIENCE

Application Report

Qube Opto 384 - Optical modulation of ion channels

High throughput automated patch clamping with optogenetic capabilities can modulate ion channels through optogenetic actuators or light activated compounds.

Summary

Using the optical features of Qube Opto 384, it is possible to evaluate both light-activated ion channels and photoactivated ligands. Here we outline a few of the studies we have performed on Qube Opto 384.

- Channelrhodopsin 2 (ChR2) could be activated and the channelrhodopsin 2 mediated current could be manipulated in both a light and voltage-dependent manner, with activation times as short as 4 ms. A light-induced chloride current could also be elicited, employing the chloride-conducting channelrhodopsin iC++.
- Caged γ-aminobutyric acid (Rubi-GABA) could be activated by light and give rise to a $GABA_AR$ mediated current. The GABA response was both concentration and the light intensity dependent. The optical activation of GABA combined with microfluidic channels also enabled ultra-short ligand exposure times.

Introduction

Qube Opto 384 is a full scale, integrated high throughput screening (HTS) system

- Optical stimulation combined with automated patch clamping (APC)
- 384 well format
- Fully software controlled by Qube software
- Dark cabinet allows for automated handling of light-sensitive compounds and cells
- Compatible with other Qube features such as temperature control

Qube Opto 384, features

- Addressing 384 sites simultaneously
- Individual light control of 24 columns
- Software controlled
- Variable timing, duration and intensity of light
- Light ramps and other optical waveforms
- Independent of amplifiers and robot – i.e. flexibility with respect to assay design

Optogenetics

Optical stimulation combined with automated patch clamp allows for control of light activated channel and compounds in combination with classical electrophysiology. Genetically engineered ion channels and receptors activated by light can be expressed in mammalian cells, a technique often referred to as optogenetics. Since the recent employment of channelrhodopsin (ChRs), a photoreceptor type found in green algae (Nagel et Al., 2002), there has been an explosion in the development of new light activated proteins, now including a broad selection of receptors, enzymes and ion channels (Jiang et Al., 2017).

Optical stimulation and automated patch clamp can also take advantage of photochemistry, where light-modulated chemical structures can be used to evaluate pharmacological properties, a technique also called optopharmacology.

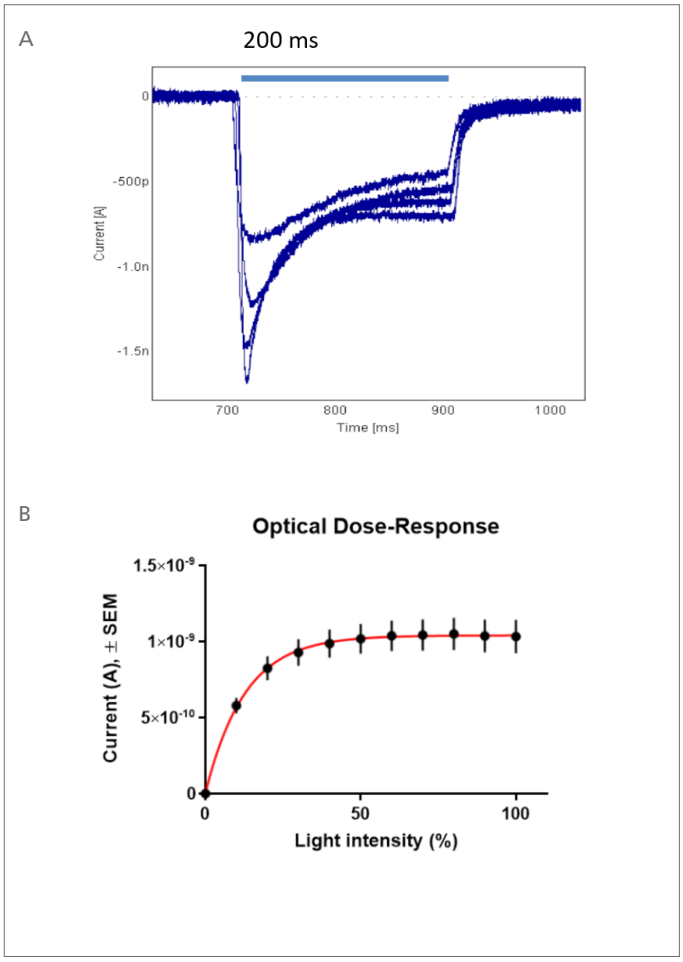
Results and discussion

Optogenetics

Optogenetics combines genetic manipulation and optics offering unprecedented opportunities for control of cells by light and provides millisecond-scale temporal precision which allows the experimenter to keep pace with fast biological information processing. ChRs are currently extensively used for control of cells by light. ChRs are light-sensitive, non-selective cation channels permeable to Na^+, K^+ and Ca^{2+} and when opened upon illumination they depolarize the membrane (Volkov et Al., 2017). Light-driven, hyperpolarizing ion pumps are also available, e.g. Halorhodopsins, where Cl^- ions are pumped across the cell membrane powered by light.

Channelrhodopsin 2

Combined optical and voltage control over Channelrhodopsin 2 (ChR2), a light activated ion channel: HEK293 cells permanently transfected with ChR2 were patched and optically stimulated. The ChR2 mediated current could be manipulated in both a light and voltage-dependent manner.



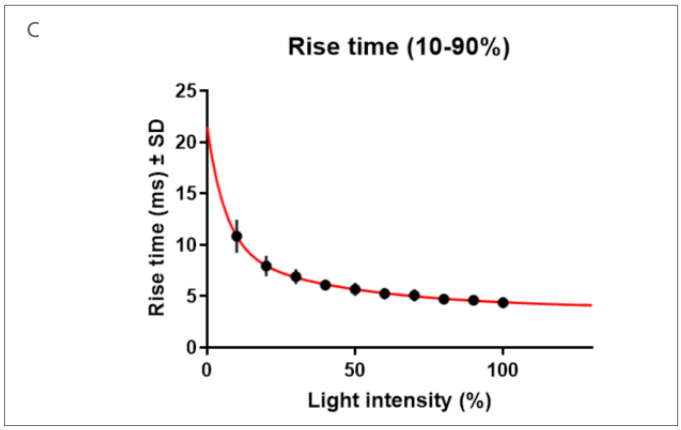


Fig. 1: ChR2: Optical dose-response relationship.
Light intensity-dependent activation of ChR2. A) Typical light-evoked current at LED output intensity 10, 20, 40 and 100 % and a holding potential of -90 mV. B) Dose-response relationship for light from 0 to 100 %. C) Fast activation kinetics: Rise time calculated as time from 10 – 90 % of maximal current amplitude. Data are represented as mean ± SD of n=12.

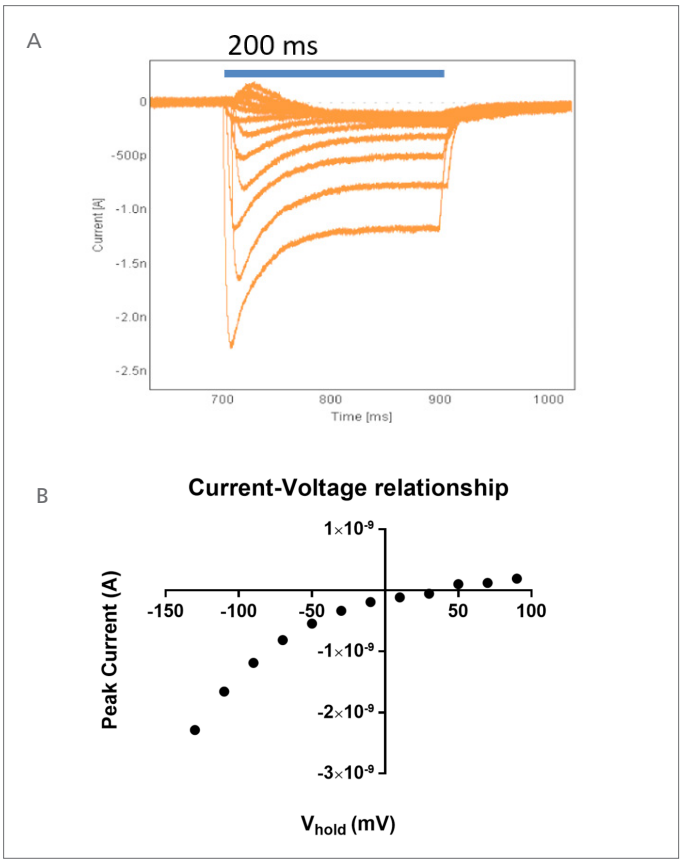


Fig. 2: ChR2: Current-Voltage relationship.
A) Typical light-evoked current at varying holding potentials. The stimulation pulse was 200 ms and the intensity 100 %. The holding potential was increased with 20 mV between sweeps, starting at -130 mV and stepwise reaching 90 mV. B) IV plot: The maximum current amplitude plotted against holding potential. Note that the ChR2 mediated current showed an inward rectification.

Chloride-conducting channelrhodopsin

Furthermore, we employed the chloride-conducting channelrhodopsin iC++, which was developed from a non-selective cation conducting channelrhodopsin through a mutational approach (Govorunova et Al., 2015). HEK293 cells permanently transfected with iC++ were patched and optically stimulated. The iC++ mediated current could be manipulated in both a light and voltage-dependent manner.

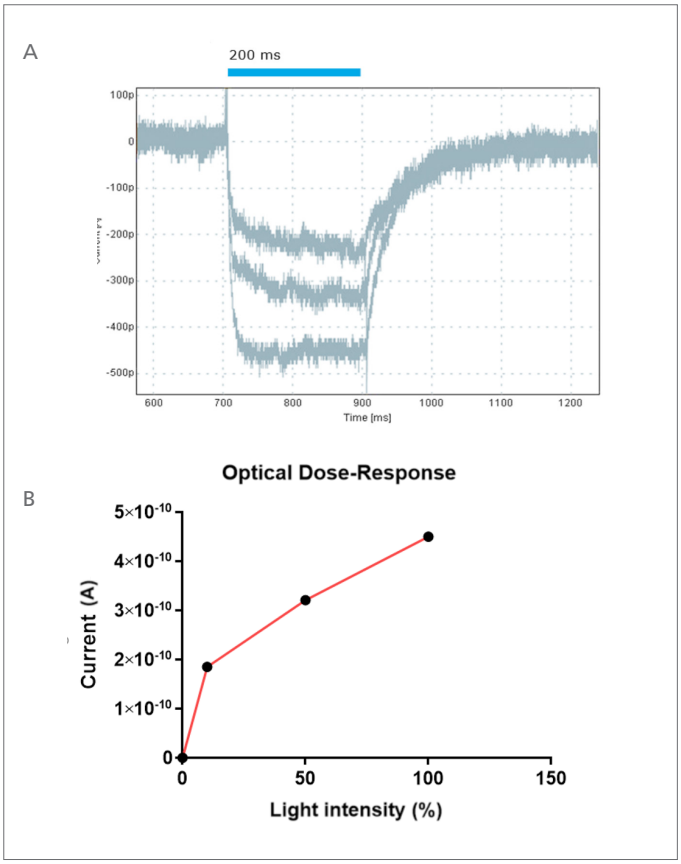


Fig. 3: Optical dose-response relationship of Cl--selective channelrhodopsin (iC++).
A) Example of iC++ mediated currents: Typical sweep plot when exposed to a 200 ms pulse of varying intensities: 10 %, 50 % and 100 %. The holding potential was clamped at -100 mV. B) Dose-response relationship for the peak current of the traces seen in (A).

Optopharmacology

Optopharmacology is work done with compounds that are activated or deactivated in response to a light stimulation. Compound activation by light enables the pharmacological manipulation of receptors, ion channels and other proteins with a high degree of temporal control (Zayat et Al., 2003). The compound can be a caged compound, which is a large construct cleaved by light, releasing an active ligand, or a photoswitchable ligand, which switches into an active conformation upon light stimulation and reverses conformation to the original state once light is removed.

Rubi-GABA is a caged GABA compound activated by visual wavelengths (Rial Verde et Al., 2008) available from Tocris. When activated by light, a $GABA_AR$ mediated currents were elicited due to the optical uncaging of GABA. The GABA response was both depending on the rubi-GABA concentration and the light intensity.

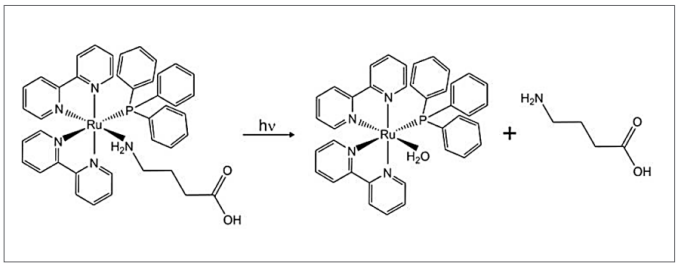


Fig. 4: GABA photorelease from ruthenium-bipyridine-triphenylphosphine-GABA (RuBi-GABA). From: Rial Verde et al., 2008.

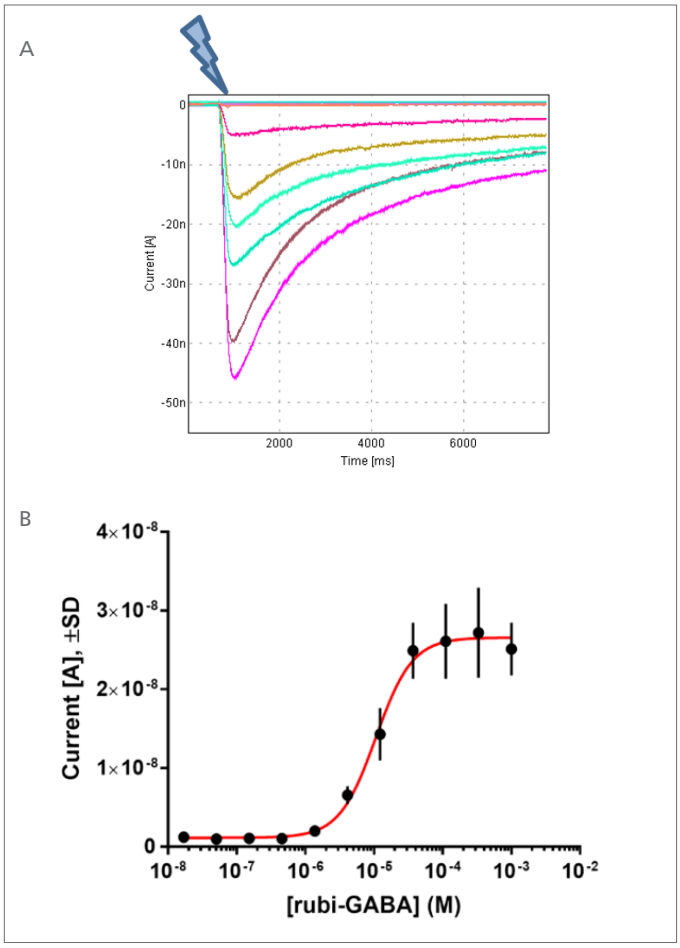


Fig. 5: Concentration-response relationship.
A) HEK 293 cells expressing $GABA_A$ ($\alpha_5\beta_3\gamma_2$) were exposed to varying concentrations of Rubi-GABA (Tocris). GABA was photo-released upon a 200 ms light pulse (475 nm) at 100 %. B) Non-cumulative concentration-response curve for Rubi-GABA of a 3-fold dilution series. A Hill equation was fitted to the data and the calculated EC_{50} was 11 µM±0.5 µM. n=32.

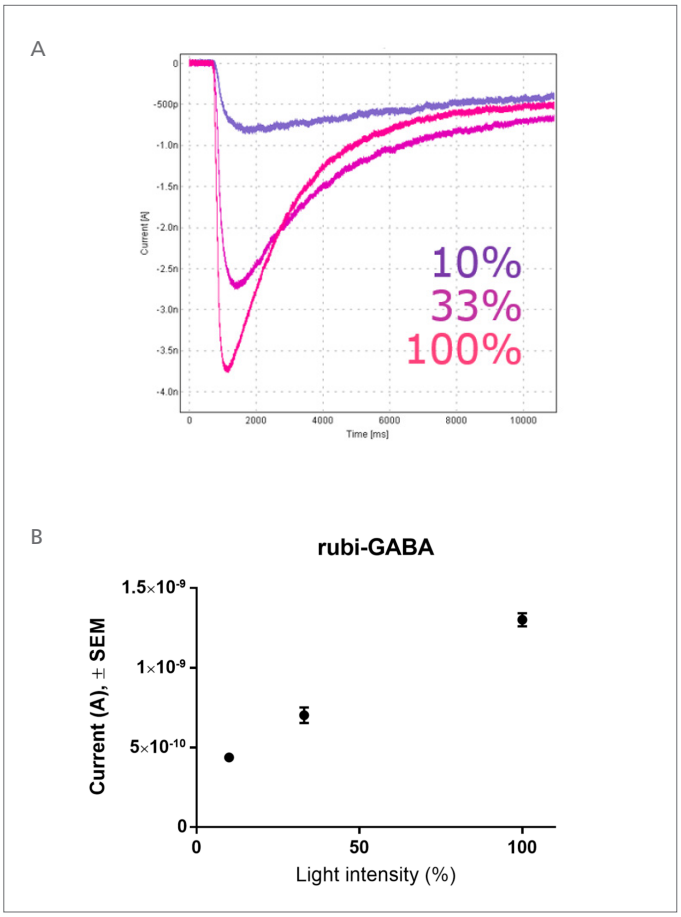


Fig. 6: Optical dose-response relationship.
A) $GABA_A$ ($\alpha_5\beta_3\gamma_2$) expressing cells were exposed to 1 mM Rubi-GABA and GABA were photo released by 3 different intensities of light. The duration of the pulses was 200 ms and the wavelength 475 nm. B) Intensity-response relationship for Rubi-GABA at intensities of 10, 33 and 100 %. n=32.

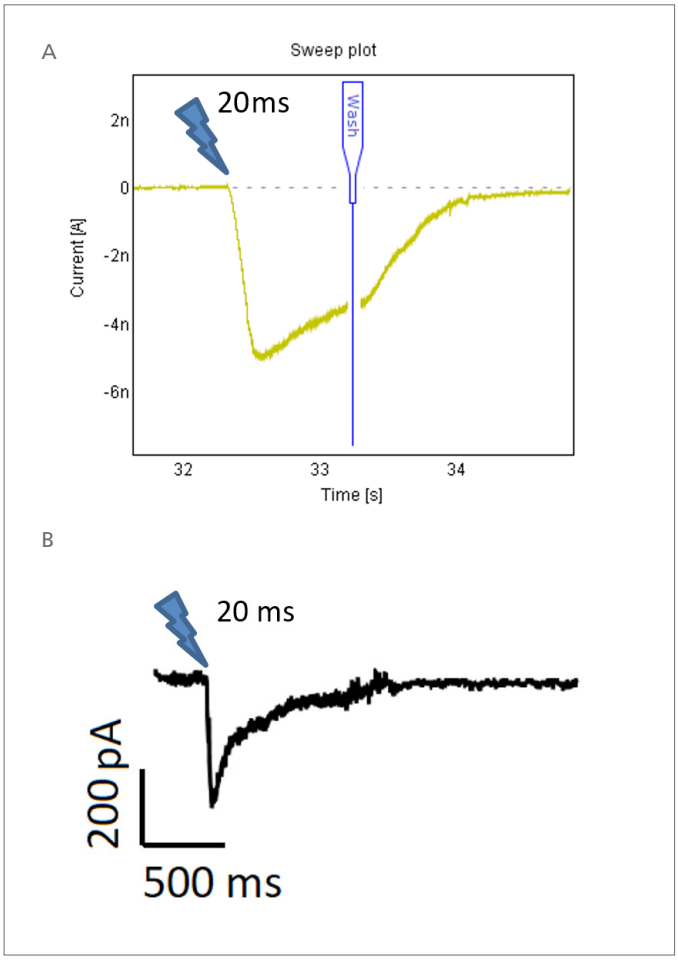


Fig. 7: Short ligand exposure time
A) RuBi-GABA activation followed by wash-out
When 200 µM RuBi-GABA is washed in, no response is seen, as the caged GABA does not activate the GABA receptors. Upon stimulation with light for 100 ms, GABA is uncaged and activates the $GABA_A$ receptor. 500 ms after the stimulus, the microfluidic channel is flushed, and the response terminated.
Compound consumption: 7 µL/site.
B) RuBi-GABA activation during perfusion
To further decrease the ligand exposure time, the light stimulation (20 ms) was executed during perfusion with RuBi-GABA (330 µM). The constant perfusion resulting in an instantaneous wash-out of uncaged GABA and in combination with a shortening of the light stimulus, a sharp, ultrashort GABA response could be recorded.
Compound consumption: 7 µL/site.

Short ligand exposure time on Qube Opto 384

With the new optical capability of Qube Opto 384 in combination with the microfluidic system of the QChip 384, the exposure time of a ligand can be drastically reduced. Here we use rubi-GABA, a caged GABA compound, and demonstrate two strategies to obtain short ligand exposure (see Fig.7).

Methods

ChR2/HEK 293, $GABA_A(\alpha_5\beta_3\gamma_2)$/HEK 293, and iC++/HEK 293 cells were cultured according to the supplier's description. ChR2 and $GABA_AR$ were stably expressed in the HEK cells whereas iC++ was induced 24 h prior to the experiment. All experiments were carried out at ambient temperature using QChip 384X multi-hole consumables and patched using a standard whole-cell protocol.

Note: Qube Opto 384 is currently launched as an in-house capability at Sophion Bioscience. For more information please contact Sandra Wilson - swi@sophion.com.

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